Effect of vitamin-enriched bread on the vitamin status of an isolated rural community — a controlled clinical trial

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Malnutrition is an inclusive term that entails the lack, excess or imbalance of one or more nutrients required to maintain normal nutritional status and optimal health. Malnutrition affects a large segment of the world's children.[1] It is estimated that the prevalence of childhood malnutrition in South Africa ranges from 5% to 50% of the current population.[2] It is well documented that an inadequate intake of micronutrients causes deficiency disorders and that such deficiencies often accompany malnutrition.[3] The dietary intake of calcium, iron, zinc, folic acid, riboflavin, vitamin B_6 and vitamin C has been reported to be low, in comparison with the recommended dietary allowances (RDA),[4] in a significant proportion of children in the country; it is often lower than 67% of the RDA, indicating a population at risk of deficiency.[5,6] In adults 15 - 64 years of age, the dietary intake of such micronutrients as vitamin B_6, iron, calcium, magnesium, zinc, copper and folic acid has also been reported to be significantly lower than the RDA.[5,6]

Fortification of food staples has been successfully implemented in a number of countries to increase the dietary intake of micronutrients.[7] Fortification can be rapidly implemented, is flexible as well as cost-effective and is socially acceptable. When correctly introduced and controlled, it can improve the dietary intake of a given population. Evidence supporting the success of this particular type of nutritional intervention includes the addition of iodine to salt in the prevention of endemic goitre and the fortification of cow's milk with vitamin D, which has been considered to be the major factor in the disappearance of infantile rickets.[4,8] Further, the addition of thiamin, riboflavin, nicotinic acid and calcium to cereal grain products such as flour and maize meal, as well as the addition of vitamin A, vitamin B_6, folic acid, magnesium, and zinc to

special formula bread, has been credited with substantial increases in blood levels of these nutrients in the population of the United States.[9,10]

Cereal grain products are reported to provide 52% of the average global per capita intake of energy; further, in developing countries, cereal grain products can provide up to 70% of the daily energy intake. Because cereal products enjoy such wide acceptability as daily staple foods, they have been used successfully as base carriers for fortification.[11,12] In South Africa, the milling industry has voluntarily enriched maize meal with riboflavin and niacin. However, the monitoring of this practice is inadequate[13] and its impact is largely unknown.[14]

The available evidence in South Africa, albeit fragmented, indicates that micronutrient deficiencies do occur.[14] In this regard SASKO, a major food manufacturer in the country, introduced an enriched bread range, so that a 250 g portion of bread would supply 25% of the RDA for thiamin, riboflavin, niacin, pyridoxine, folic acid and calcium. This study was undertaken before the enriched bread was launched with a view to evaluating the effect of its consumption on the vitamin status of a rural community.

Subjects and methods

Study population

Since SASKO's enriched bread would provide only 25% (per 250 g portion) of the RDA for the said vitamins, it was deemed necessary to study a population known to have an inadequate vitamin intake and a poor vitamin status. Bushmen residing at Kagga Kamma have been shown[15] to meet these prerequisites. The diet of this community lacks variety and consists mainly of bread; as such, the introduction of SASKO's enriched bread would not have interfered in any way with their diet or impacted adversely on their lifestyle. This population was therefore studied after extensive consultation with the members of the community.

Subjects

The purpose of the study was explained to the subjects in a community meeting and the agreement of the subjects to participate in the study was unanimous. The study was approved by the Ethics Committee of the University of Stellenbosch. Bushmen of both sexes, older than 10 years of age and known to have subnormal blood levels of at least 2 or more of the vitamins added to the enriched bread, were included in the study. Twenty-nine subjects, out of 40 members of the community, aged 10 - 95 years met these criteria and were randomly allocated to receive either the enriched standard bread (N = 15; test group) or the standard bread (N = 14; control group). To ensure blindness of the study, only 2 of the investigators (D.L., I.L.) and the manager of the bakery had the key to the bread type code. At the end of the 4-month period, 6 of the subjects (4 consuming the enriched and 2 the standard bread) dropped out of the study because they went to the Kalahari, thus leaving 11 and 12 subjects in the enriched and standard bread group, respectively.

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Bread

To ensure compliance, sensory tests had been done with enriched and standard bread before the beginning of the study to assess any differences in the appearance of the bread, its taste, texture and aroma. Both types of bread were equally acceptable and no differences were detected by the subjects. Additionally, to prevent discontent in the community, those members of the community who did not participate in the study were also provided with the standard bread. Subjects were asked to eat only the bread specifically provided to them, even within the same household.

The bread (400 g per person per day) was individually packed in plastic bags, appropriately labelled and delivered to each household 3 times a week to ensure its freshness and prevent deterioration of quality and composition due to storage. The bread was consumed daily for a period of 4 months. As a supplementary investigation, the subjects were studied for a further month during which only 200 g of bread per day was given; however, during this extra month of observation, the enriched bread contained double the amount of the added vitamins so as to provide the same level of vitamin intake. This was done because some subjects had difficulty, in the later part of the study, in consuming 400 g of bread per day. Bread not consumed, together with empty plastic bags, was collected the following day, weighed (to the nearest 0.01 kg) on a digital scale and removed by the supplier at the next bread delivery. A daily record of wastage was kept for each subject. The plastic bags served as an indication that the subject had consumed his or her portion of bread the previous day and gave a measure of compliance. Random duplicate portions of bread (N = 9) were analysed (Spillers Premium P, England) for their vitamin content once weekly for the duration of the study.

Dietary intake

A dietitian (W.B.) was employed specifically for the study and lived on site, with only 3 brief periods of absence. She was responsible for the dietary and anthropometric assessment as well as the monitoring of bread distribution and the recording of bread wastage. She was unaware of the bread type code. A dietary history was obtained from each participant prior to the initiation of the study, to confirm previously determined[15] dietary patterns and intake. Food consumption patterns were then monitored and recorded on a monthly basis, to determine any deviation from the diet consumed before the study (with the exception of the SASKO bread). To determine dietary intake, the 24-hour recall method was employed on 3 consecutive days (weekends not excluded) in a month, using structured and validated questionnaires. Quantities were determined using household measures familiar to the respondents. The mean daily intake of nutrients was analysed using the South African food tables.[16] Qualitative information included storage facilities and availability of food throughout the year.

Anthropometric assessment

Body weight (to the nearest 0.1 kg) was determined using electronic scales (Sauter, SA, Ltd) once monthly. Subjects were weighed wearing only their traditional loin cloths.

Vitamin status

Blood was drawn from the antecubital fossa by a doctor or a nursing sister with intensive care training. A blood sample (10 ml) was drawn from each subject prior to the initiation of the study and again monthly thereafter until the end of the study. The blood was protected from light at all times and kept in cool boxes containing ice packs; the samples were coded and transported to the laboratory where they were processed. All operations were completed within a maximum of 12, usually 8, hours. Whole blood/red blood cells/serum or plasma, as appropriate, was used for the determination of thiamin,[17] riboflavin,[18] niacin,[19] vitamin B_6[20] and folic acid.[21] None of the laboratory personnel knew the sample codes.

Statistical analysis

Student's independent t-test was used for comparing the two groups. Although one-tailed t-tests are justified on the basis of the study design, the more conservative two-tailed t-tests are reported, except where indicated otherwise. Comparisons were carried out in respect of mean values as measured at the end of each observation period. For blood vitamin values, the *mean change* in these values (average of the last 4 observations minus baseline for each subject) was also compared over the period of the study. Values are tabulated as the mean and standard deviation (SD). Values of $P \leqslant 0.05$ were considered statistically significant.

A linear discriminant analysis on the *mean change* in all the blood vitamin values, considered jointly, was used for assessing the relative importance of the various values in identifying differences between the two groups and for predicting the classification of the subjects in terms of the type (enriched or standard) of bread consumed over the study period.

Results

Body weight

Although the subjects were allocated randomly to the two groups, the test group had a lower mean body weight at baseline than the control group (Table I). This difference, which was not statistically significant, was however maintained throughout the study period. Body weight remained constant in both groups of subjects throughout the study period. The maximum individual fluctuation in body weight in one subject was a 3 kg weight loss which was regained.

Table I. Mean body weight (kg) at baseline and at the end of each observation period (mean and SD)

Point of observation	N	Control group	N	Test group
Baseline	14	40.0 (8.8)	15	36.7 (8.3)
1 month	14	39.7 (8.3)	15	36.0 (8.3)
2 months	14	39.6 (8.5)	15	36.0 (7.6)
3 months	14	38.9 (8.5)	15	35.0 (8.2)
4 months	12	40.0 (9.1)	11	36.1 (7.8)

Nutrient intake

Energy and vitamin intake derived from sources other than bread did not differ significantly at the end of each observation period between the two groups (Table II). Although bread consumption was significantly ($P < 0.05$) higher for the test group at the second and third observation point (Fig. 1), it did not influence the total energy intake between the two groups significantly (Table III). The vitamin intake from all sources including bread was, however, significantly ($0.01 < P < 0.05$) higher in the group consuming the enriched bread (Table III). The mean concentration of the vitamins added to the bread in the random duplicate portion analyses ($N = 9$) was above the desired level of enrichment, namely 25% of the RDA per 250 g portion (thiamin 0.67, SD 0.11 mg (range 40 - 56% of the RDA), vitamin B_6 0.62, SD 0.04 mg (range 29 - 33% of the RDA), niacin 8.3, SD 0.58 niacin equivalents (range 42 - 49% of the RDA) and folic acid 78, SD 17 µg (range 30 - 48% of the RDA));
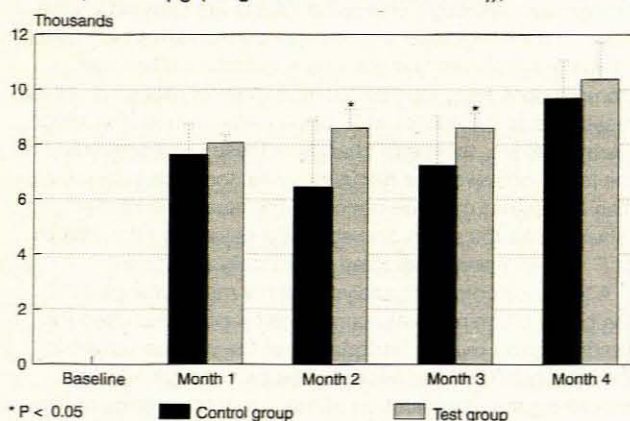

Fig. 1. SASKO bread consumption (g) at baseline and at the end of each observation period (mean and SD).

corresponding data for riboflavin were considered unreliable due to methodological problems in the analyses and are therefore not reported; nevertheless, available data indicated an enrichment range of 14 - 30% of the RDA.

Blood vitamin values

The mean serum concentration of vitamin B_6 (pyridoxal-5-phosphate) was significantly ($P < 0.05$) higher at the last point of observation (3.1, SD 1.6 v. 4.5, SD 2.0 ng/ml for the control and test group respectively). Similarly, serum folic acid was significantly ($P < 0.05$) higher at the second point of observation in the group consuming the enriched bread (2.9, SD 1.0 v. 4.0, SD 1.6 ng/ml for the control and test group respectively). For both of these vitamins as well as for thiamin (thiamin pyrophosphate (TPP) effect), blood values in the test group were consistently higher (lower in the case of the TPP effect), albeit not significantly so, at all observation points during the study.

The *mean change* (average of the last 4 observations minus baseline for each subject) in serum values, adjusted for bread intake ((average of last 4 observations minus baseline/mean daily bread intake) x 100 for each subject), of serum vitamin B_6 and serum folate, as well as red blood cell folate and TPP effect (one-tailed t-test) was significantly greater (Fig. 2) in the group consuming the enriched bread over the 4-month study period ($0.01 < P < 0.05$). The findings during the supplementary period of observation confirmed those of the main study.

In the linear discriminant analysis, blood levels of vitamin B_6 and folate were found to be the most important variables. Discriminant functions based on all the blood vitamin values correctly identified all but 2 subjects in the appropriate group. This represents an 86% reduction over the classification accuracy that could be expected if the observations were randomly classified. The probability of a

Table II. Daily energy and vitamin intake (excluding bread intake) at baseline and at the end of each observation period (mean and SD)

Point of observation	Energy (kJ) Control group	Energy (kJ) Test group	Thiamin (mg) Control group	Thiamin (mg) Test group	Riboflavin (mg) Control group	Riboflavin (mg) Test group	Niacin (NE)* Control group	Niacin (NE)* Test group	Vitamin B_6 (mg) Control group	Vitamin B_6 (mg) Test group	Folate (µg) Control group	Folate (µg) Test group
Baseline	2 400 (586)	2 338 (722)	0.31 (0.13)	0.33 (0.18)	0.96 (0.23)	0.90 (0.23)	2.98 (1.51)	2.60 (1.13)	0.49 (0.21)	0.50 (0.19)	48.93 (18.24)	47.40 (13.08)
1 month	1 898 (449)	1 784 (316)	0.15 (0.05)	0.21 (0.16)	0.31 (0.12)	0.32 (0.16)	4.09 (2.11)	3.42 (1.20)	0.18 (0.11)	0.25 (0.20)	22.93 (13.66)	25.67 (12.73)
2 months	2 160 (499)	2 395 (482)	0.16 (0.04)	0.21 (0.07)	0.31 (0.12)	0.35 (0.11)	5.25 (2.72)	4.78 (1.69)	0.32 (0.18)	0.35 (0.14)	29.20 (08.38)	31.87 (14.65)
3 months	2 319 (529)	2 226 (533)	0.22 (0.07)	0.19 (0.07)	0.23 (0.07)	0.32 (0.16)	4.18 (1.70)	4.04 (2.54)	0.34 (0.12)	0.27 (0.16)	28.50 (09.57)	25.27 (16.79)
4 months	3 708 (932)	3 583 (453)	0.37 (0.11)	0.34 (0.08)	0.39 (0.13)	0.38 (0.15)	7.48 (3.41)	6.91 (3.51)	0.41 (0.13)	0.32 (0.14)	40.77 (20.12)	30.73 (7.68)

*NE = niacin equivalent.

Table III. Daily energy and vitamin intake (including bread) at baseline and at the end of each observation period (mean and SD)

Point of observation	Energy (kJ) Control group	Energy (kJ) Test group	Thiamin (mg) Control group	Thiamin (mg) Test group	Riboflavin (mg) Control group	Riboflavin (mg) Test group	Niacin (NE)* Control group	Niacin (NE)* Test group	Vitamin B_6 (mg) Control group	Vitamin B_6 (mg) Test group	Folate (µg) Control group	Folate (µg) Test group
Baseline	2 400 (586)	2 338 (722)	0.31 (0.13)	0.33 (0.18)	0.96 (0.23)	0.90 (0.23)	2.98 (1.51)	2.60 (1.13)	0.49 (0.21)	0.50 (0.19)	48.93 (18.24)	47.40 (13.08)
1 month	4 874 (973)	5 053 (457)	0.57 (0.08)	0.97 (0.16)†	0.47 (0.11)	0.51 (0.16)†	8.33 (2.02)	12.55 (1.92)†	0.39 (0.09)	1.04 (0.21)†	80.08 (22.49)	100.6 (14.6)†
2 months	5 024 (901)	5 509 (1110)	0.55 (0.05)	0.96 (0.10)†	0.47 (0.12)	0.54 (0.12)†	10.23 (3.33)	13.56 (2.04)†	0.52 (0.18)	1.12 (0.16)†	81.34 (9.04)	104.2 (16.58)†
3 months	4 741 (908)	5 087 (985)	0.52 (0.10)	0.83 (0.09)†	0.35 (0.09)	0.48 (0.16)†	7.32 (2.1)	11.52 (2.78)†	0.49 (0.12)	0.93 (0.14)†	69.28 (12.01)	88.07 (18.63)†
4 months	5 741 (848)	5 685 (795)	0.65 (0.11)	0.90 (0.12)†	0.50 (0.13)	0.95 (0.12)†	10.35 (3.43)	13.82 (2.94)†	0.55 (0.13)	0.98 (0.12)†	78.23 (18.81)	158.73 (18.2)†

*NE = niacin equivalents.
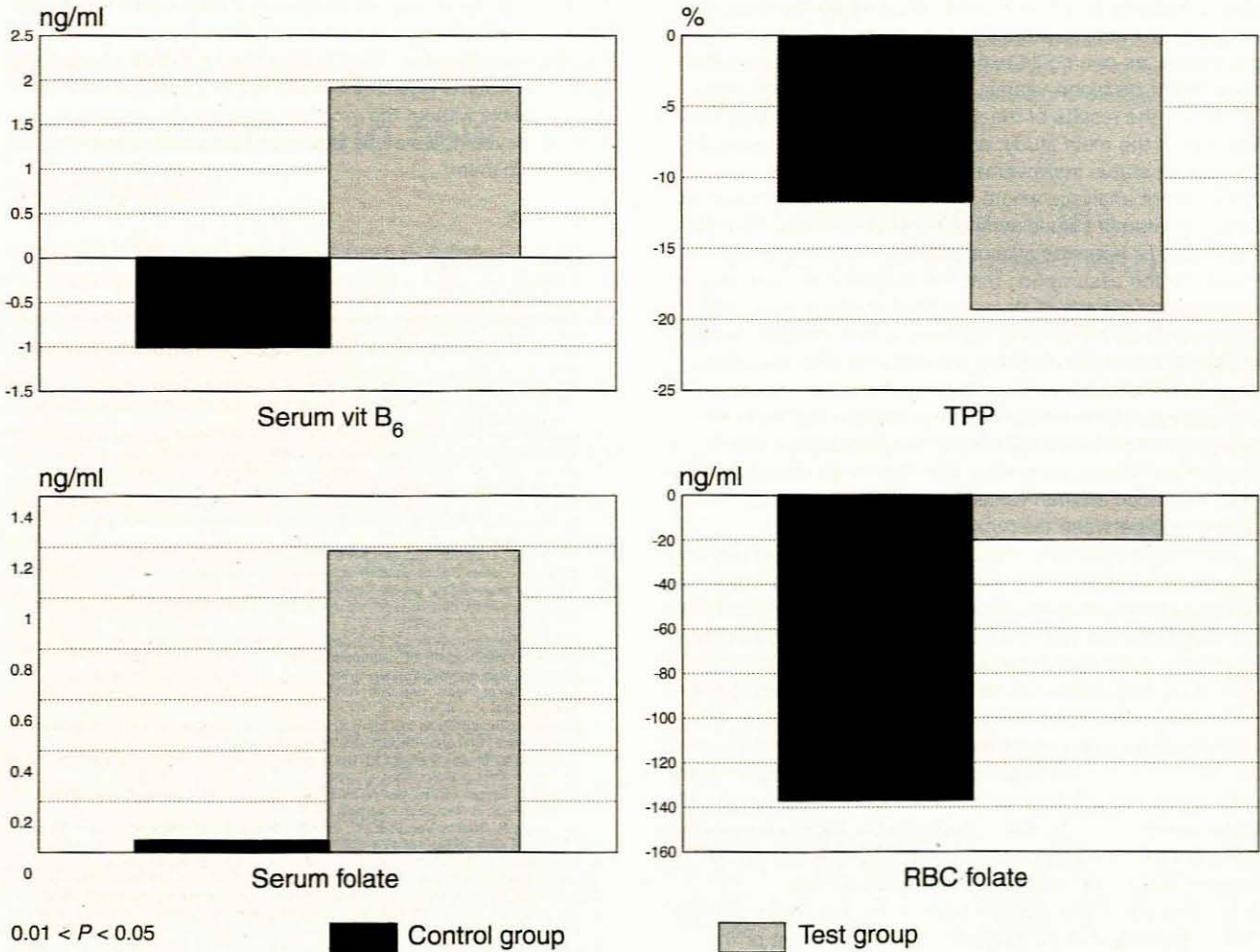† Statistically significant at the 5% or lesser level.

Fig. 2. Mean change in plasma values over study period standardised by mean daily SASKO bread intake.

subject from the enriched bread group being correctly identified was high and that of a subject from the control group being classified in the enriched group was generally very low (Fig. 3).
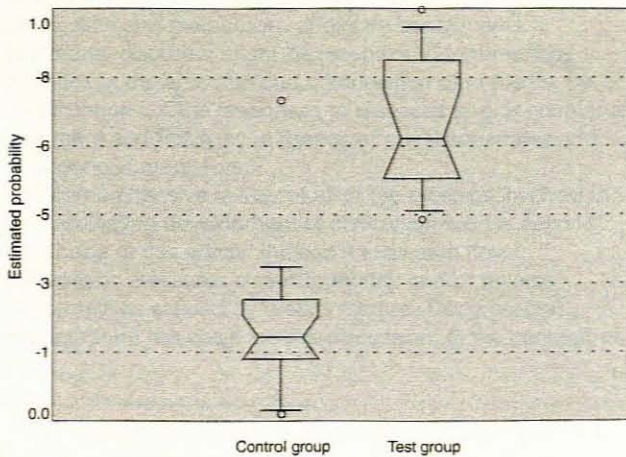

Fig. 3. Box plot of probability of being classified in the test group.

Discussion

In this study, the consumption of a specified amount of vitamin-enriched bread has been shown to improve overall the previously poor vitamin status of the rural community studied. Although the study group was small, it was nevertheless isolated and therefore had very little access to other foodstuffs besides those sold in the on-site shop at Kagga Kamma. This environment made the community an ideal population to study for a number of reasons. Firstly, the prevailing environment would minimise outside influences in terms of food consumption patterns and of the introduction of new foods. Secondly, even in the event of this occurring, it could be reasonably well monitored from the records kept at the store; and thirdly, this community was known to have a poor vitamin status. These were very important considerations in view of the comparatively small (25% of RDA) level of bread enrichment with the 5 vitamins.

It is interesting that not all the blood values determined showed a significant change. In this regard the small number of subjects should be borne in mind. Nevertheless, a tendency for an overall improvement in the vitamin status was indeed apparent. This impression is supported, firstly, by the *mean change* in blood values; it is important to

realise that this parameter gives equal weight to each study period and it therefore results in a conservative test procedure, as one could expect the effect of bread vitamin enrichment on blood vitamin values to increase with time. Secondly, the results of the supplementary study confirm the findings of the main study; and, thirdly, additional support for vitamin status improvement is provided by the discriminant analysis, where the composite contribution of all blood vitamin values (whether shown to be statistically significant or not) was evaluated. Discriminant analysis is based on the assumption that the independent variables in each group follow a multivariate normal distribution with equal variance-covariance matrices across groups. Although studies have shown that this technique is fairly robust to departures from either assumption,[22] the discriminant analysis should be interpreted with caution and seen as being confirmatory to the t-tests. Nevertheless, it does provide an interesting graphical indication of the information in all the blood vitamin values concerning the overall differences between the two groups.

Apart from statistical considerations and despite the long-term nature of the study, it is well known that the expected temporal changes in blood concentration of vitamins differ not only between vitamins but also with the supplementary doses administered. For instance, serum folic acid and vitamin B_6 concentrations as well as the TPP effect (all of which improved significantly in the group receiving the enriched bread) reflect recent dietary intake. Within the limitations of the methodology employed, other parameters indicative of longer-term status, for instance red blood cell transketolase activity, may require longer supplementation periods to elicit a response at the supplementary doses present in the fortified bread.[23] Alternatively, the bioavailability of the vitamins used in the enrichment of the bread, such as that of riboflavin, may be different or the methodology employed may be insufficiently sensitive to detect smaller changes. Compliance should also be considered. Although the overall compliance was good, it did transiently decrease at times, especially towards the end of the 4-month period. Furthermore, approximately 2 months after the initiation of the study the majority of the community developed the common cold. The latter was fairly severe in some subjects, requiring hospitalisation because of respiratory complications of one of the subjects in the group consuming the standard bread. In this regard it is well described[24] that the presence of the acute phase response significantly decreases blood vitamin concentrations, an effect which may have attenuated the improvement in vitamin status.

In conclusion, consumption of the enriched bread appears to offer significant benefits in terms of improving vitamin status, and it would appear to be effective in increasing dietary vitamin intake and preventing vitamin deficiencies in subjects with poor vitamin status.

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